

## REMARKS

### I. Status Summary

Claims 1-4, 6-14, 36, 38, 39, and 41-47 are now pending in the subject U.S. patent application and have been examined by the United States Patent and Trademark Office (hereinafter "the Patent Office").

Claims 42-45 are subject to certain formal objections.

Claims 1-3, 6-9, 36, 38, 39, and 41-47 have been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over McMahan *et al.* (1996) 236 *Anal Biochem* 101-106 (hereinafter "McMahan") or the Molecular Probes Technical Literature of record (hereinafter "Molecular Probes") in view of U.S. Patent No. 7,183,392 to Wagner et al. (hereinafter "Wagner"), Chaga *et al.* (2001) 49 *J Biochem Biophys Meth* 49:313-334 (hereinafter "Chaga"), and Zachariou *et al.* (2000) 890 *J Chromatog A* 890:95-116 (hereinafter "Zachariou").

Claims 1, 2, 4, 6-14, and 41-46 have also been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over Ehteshami (1996) Synthesis and Characterization of Bioaffinity Interactive Heterobifunctional Polyethylene Glycols, Ph.D. dissertation, University of Arizona (hereinafter "Ehteshami"), as evidenced by Ehteshami *et al.* (1996) 9 *J Mol Recog* 733-737 (hereinafter "Ehteshami et al.") in view of Chaga and Zachariou.

Claims 36, 37-39, and 47 have been rejected under 35 U.S.C. § 103(a) upon the contention that the claims are unpatentable over Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou, and in further view of Molecular Probes.

Claims 10 and 42-45 have been amended. The amendments to these claims are formal in nature and are limited to correcting a typographical error (claim 10) and updating the dependency of claims 42-45. Support for the amendments can be found throughout the specification as filed, including particularly in the claims as originally filed. Additional support can be found in the specification at page 9, lines 18-24. Accordingly, no new matter has been added by the amendments to the claims.

Reconsideration of the application as amended and based on the remarks set forth herein below is respectfully requested.

II. Response to the Objections to the Claims

Claims 42-45 are subject to certain formal objections. Particularly, claims 42-45 have been objected to because they depend from a cancelled claim. As set forth in the Non-Final Official Action, applicant's representative Christopher P. Perkins confirmed in a telephone conference on January 23, 2008 with Examiner Fetterolf that claims 42-45 should in fact be dependent from claim 41. Claims 42- 45 have been amended to depend from claim 41. Support for the amendments can be found throughout the specification as filed, including particularly in the claims as originally filed. Additional support can be found in the specification at page 9, lines 18-24. Thus, the instant objections are believed to be moot.

Accordingly, applicant respectfully submits that the objections to claims 42-45 be withdrawn at this time.

III. Responses to the Rejections under 35 U.S.C. § 103

Claims 1-3, 6-9, 36, 38, 39, and 41-47 Have been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over McMahan or Molecular Probes in view of Wagner, Chaga, and Zachariou. Claims 1, 2, 4, 6-14, and 41-46 have also been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou.

After careful consideration of the rejections and the Patent Office's bases therefore, applicant respectfully traverses the rejections and submit the following remarks.

III.A. Response to the First Obviousness Rejection

Claims 1-3, 6-9, 36, 38, 39, and 41-47 have been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over McMahan or Molecular Probes in view of Wagner, Chaga, and Zachariou. The Patent Office contends that McMahan discloses a conjugate comprising an NTA polydentate chelator and a biotin detectable moiety conjugated to the polydentate chelator. The chelator is asserted to be conjugated to Ni<sup>2+</sup>. Molecular Probes is asserted to disclose a conjugate of the formula Biotin-X NTA comprising a chelator-metal ion moiety and a detectable

moiety conjugated to the chelator-metal ion moiety. Again, the chelator is asserted to be conjugated to  $\text{Ni}^{2+}$ . The Patent Office concedes, however, that neither McMahan nor Molecular Probes teaches that the chelated metal ion is  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , or  $\text{Ga}^{3+}$ , or that the binding solution is in a pH range of 5 to 7.0.

These deficiencies are asserted to be cured by Wagner, Chaga, and Zachariou. According to the Patent Office Wagner teaches nitrilotriacetic acid coordinated with metals such as Ni, Co, Fe, and Cu bind His-tags of from 6 to 9 amino acids. Chaga is asserted to teach IMAC as a separation technique that utilizes the differential affinity of proteins for immobilized metals to effect their separation, wherein hard metals such as  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$  show preference for oxygen, soft metals such as  $\text{Cu}^+$  and  $\text{Hg}^{2+}$  prefer sulfur, and intermediate metals such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Co}^{2+}$  coordinate to nitrogen, oxygen, and sulfur. Chaga is also asserted to teach that IMAC has seen extensive work in the purification of proteins from complex biological samples such as the use of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Zn}^{2+}$  for the purification of proteins having exposed histidine residues, as well as the use of  $\text{Fe}^{3+}$  and  $\text{Ga}^{3+}$  for the enrichment of phosphorylated proteins and peptides. Additionally, the reference is asserted to teach that immobilized  $\text{Fe}^{3+}$  would adsorb a distinct profile of proteins at acidic pH from that which would be adsorbed to immobilized  $\text{Cu}^{2+}$  at neutral pH. Zachariou is asserted to teach the binding properties of immobilized O-phosphoserine (*im*-OPS) and 8-hydroxyquinoline (*im*-8-HQ) with immobilized iminodiacetic acid (IDA) as the control system in combination with the hard Lewis metal ions,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Yb}^{3+}$ , and the borderline metal ion  $\text{Cu}^{2+}$ , over a pH range of 5.5 to 8.0. With regards to the pH, the reference teaches that with a incubation/equilibrium buffer of 0.5 M or 0.06M ionic strength, fewer proteins bound to these hard Lewis metal ion IMAC adsorbents as the pH became increasingly alkaline, which is opposite to what is observed with protein with the borderline Lewis metal ion IMAC systems.

From this, the Patent Office asserts that it would have been *prima facie* obvious to one of skill in the art to combine the teachings of the cited references to modify the heterobifunctional conjugate as taught by McMahan or Molecular Probes with  $\text{Fe}^{3+}$  in view of the teachings of Wagner. According to the Patent Office, one would have been motivated to do so because Wagner teaches that NTA coordinated with  $\text{Fe}^{3+}$  binds His-

Tags. Thus, it is contended that one of ordinary skill in the art would have a reasonable expectation of success that by modifying the heterobifunctional conjugate as taught by McMahan or Molecular Probes with  $\text{Fe}^{3+}$  in view of the teachings of Wagner one would achieve a metal chelate which recognizes a His tag.

The Patent Office further contends that it would have been *prima facie* obvious to combine the teachings of the reference so as to substitute the metal ion which coordinates to the heterobifunctional conjugate as taught by McMahan or Molecular Probes to a hard Lewis metal ion such as  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , or  $\text{Ga}^{3+}$  upon the contention that Chaga teaches that different metals such as hard Lewis metals may be successfully used for detecting various proteins such as phosphoproteins which are not detected using intermediate metals such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , or  $\text{Co}^{2+}$ .

And finally, the Patent Office asserts that it would have been *prima facie* obvious to combine the teachings of the references so as to use a lower pH binding solution when detecting proteins using hard Lewis metal ions such as  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , or  $\text{Ga}^{3+}$  because the prior art recognizes as taught by both Chaga and Wagner, that hard Lewis metals preferentially bind proteins at a lower pH than intermediate metals such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Co}^{2+}$ .

Applicant respectfully disagrees that the Patent Office has presented a *prima facie* case of unpatentability of the pending claims over the cited references. Applicant respectfully traverses the Patent Office's assertion that one of ordinary skill in the art would have been motivated to modify the heterobifunctional conjugate as taught by McMahan or Molecular Probes with  $\text{Fe}^{3+}$  in view of the teachings of Wagner because Wagner teaches that NTA coordinated with  $\text{Fe}^{3+}$  binds His-Tags. Applicant respectfully submits that both McMahan and Molecular Probes explicitly teach using  $\text{Ni}^{2+}$  for binding His tags. Applicant respectfully submits that there would be no motivation for one of ordinary skill in the art to remove the  $\text{Ni}^{2+}$  ion from the conjugates of McMahan and/or Molecular Probes and replace the same with a different ion merely in order to use the conjugates for exactly the same function that the McMahan and Molecular Probes conjugates originally had. Applicant respectfully submits that there is no advantage that can be seen in making such a modification, and thus there is no support for the contention that one of ordinary skill in the art would have been motivated to do so.

Applicant respectfully traverses the Patent Office's assertion that one of skill in the art would have been motivated to substitute the metal ion which coordinates to the heterobifunctional conjugate as taught by McMahan or Molecular Probes to a hard Lewis metal ion such as  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , or  $\text{Ga}^{3+}$  upon the contention that Chaga teaches that different metals such as hard Lewis metals may be successfully used for detecting various proteins such as phosphoproteins which are not detected using intermediate metals such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , or  $\text{Co}^{2+}$ . Applicant respectfully traverses the Patent Office's assertion regarding the instant rejection, that it would have been *prima facie* obvious to one of skill in the art to use a lower pH binding solution when detecting proteins using hard Lewis metal ions such as  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , or  $\text{Ga}^{3+}$  upon the contention that Chaga and Wagner teach that hard Lewis metals preferentially bind proteins at a lower pH than intermediate metals such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Co}^{2+}$ .

It is respectfully submitted that these contentions represent overly broad readings of these references. With respect to Chaga, applicant respectfully submits that the reference discloses "immobilized  $\text{Fe}^{3+}$  would adsorb a distinct profile of proteins at acidic pH from that which would be adsorbed to immobilized  $\text{Cu}^{2+}$  at neutral pH" (Chaga at page 320; emphasis added). Contrary to the Patent Office's assertion, this passage does not suggest that immobilized  $\text{Fe}^{3+}$  would adsorb a distinct profile of proteins at acidic pH from that which would be adsorbed to immobilized  $\text{Fe}^{3+}$  at neutral pH, and thus there is no motivation in Chaga for employing a binding solution with a pH ranging from about 5.0 to about 7.0 as recited in the instant claims. Applicant further respectfully submits that Wagner fails to cure this deficiency, and Wagner does not disclose or suggest any pH conditions that would influence binding of a chelated metal ion to a phosphorylated amino acid residue in a phosphoprotein as recited in claim 1.

And finally, applicant respectfully submits that Zachariou also fails to cure this deficiency. Applicant respectfully submits that as disclosed in Zachariou at, for example, page 96 (left column, first full paragraph) *im*-OPS is employed as a chelator moiety. Applicant respectfully submits that how *in*-OPS might behave as a chelator provides no information as to how a chelated metal ion might interact with a phosphoprotein at various pH's. Therefore, even assuming *arguendo* that Zachariou discloses that fewer proteins bound to hard Lewis metal ion IMAC adsorbents as the pH

became increasingly alkaline, this does not suggest that employing a binding solution with a pH ranging from about 5.0 to about 7.0 as recited in the instant claims will result in any beneficial effect on binding of a chelated metal ion to a phosphorylated amino acid residue in a phosphoprotein as recited in claim 1.

Summarily, applicant respectfully submits that the Patent Office has not presented a *prima facie* case of unpatentability of claims 1-3, 6-9, 36, 38, 39, and 41-47 over McMahan or Molecular Probes in view of Wagner, Chaga, and Zachariou. As such, applicant respectfully requests that the instant rejection be withdrawn at this time.

### III.B. Response to the Second Obviousness Rejection

Claims 1, 2, 4, 6-14, and 41-46 have also been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over Ehteshami, as evidenced by Ehteshami et al. in view of Chaga and Zachariou. According to the Patent Office, Ehteshami discloses a conjugate comprising IDA as a polydentate chelator moiety and a biotin detectable moiety conjugated to the IDA moiety via a PEG spacer group. The reference is also asserted to teach a method of synthesizing the conjugate, and as evidenced by Ehteshami et al., that the composition is soluble in aqueous solution.

As such, the Patent Office asserts that Ehteshami teaches a heterobifunctional conjugate comprising a polydentate chelator, a linker, and a detectable moiety. The Patent Office concedes, however, that Ehteshami does not teach that the metal ion is  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , or  $\text{Ga}^{3+}$ , or that the binding solution is in a pH range of 5 to 7.0. These deficiencies are asserted to be cured by Chaga and Zachariou discussed hereinabove.

Applicant respectfully submits that Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou fails to support a rejection of claims 1, 2, 4, 6-14, and 41-46 under 35 U.S.C. § 103(a). Applicant discussed hereinabove how Chaga and Zachariou provide no motivation for one of ordinary skill in the art to employ the recited metal ions under the recited pH conditions, and applicant respectfully submits that these deficiencies are equally present in the instant combination of references. Therefore, applicants respectfully submit that Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou fails to support a *prima facie* case of unpatentability of claim 1 under 35 U.S.C. § 103(a).

Independent claims 10 and 41 also recite that the reagent comprises a metal ion is one of  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , and  $\text{Ga}^{3+}$ , and/or that the binding solution is in a pH range of 5 to 7.0. Therefore, applicant respectfully submits that claims 10 and 41 are also believed to be distinguished over the cited combination. Claims 2, 4, 6-9, 11-14, and 42-46 all depend directly or indirectly from one of claims 1, 10, and 41, and thus are also believed to be distinguished over the cited combination. As a result, applicant respectfully requests that the instant rejection of claims 1, 2, 4, 6-14, and 41-46 be withdrawn at this time.

### III.C. Response to the Third Obviousness Rejection

Claims 36, 37-39, and 47 have also been rejected under 35 U.S.C. § 103(a) upon the contention that the claims are unpatentable over Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou, and in further view of Molecular Probes.

With respect to the instant rejection, applicant respectfully submits that Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou does not disclose or suggest the subject matter of claim 1 for the reasons set forth hereinabove. Applicant further respectfully submits that Molecular Probes does not cure the deficiencies discussed hereinabove. Particularly, applicant respectfully submits that Molecular Probes does not disclose or suggest a phosphoprotein detection reagent comprising a metal ion is one of  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Yb}^{3+}$ , and  $\text{Ga}^{3+}$ , and/or that use of a binding solution is in a pH range of 5 to 7.0.

Therefore, applicant respectfully submits that the combination of Ehteshami as evidenced by Ehteshami et al. in view of Chaga and Zachariou, and in further view of Molecular Probes fails to support a rejection of claims 36, 37-39, and 47 under 35 U.S.C. § 103(a). As a result, applicant respectfully requests that the instant rejection be withdrawn at this time.

### CONCLUSIONS

In accordance with the amendments to the claims and the remarks presented hereinabove, applicant respectfully submits that claims 1-4, 6-14, 36, 38, 39, and 41-47

are in condition for allowance, and respectfully solicits a Notice of Allowance to that effect.

Should there be any minor issues outstanding in this matter, Examiner Fetterolf is respectfully requested to telephone the undersigned attorney. Early passage of the subject application to issue is earnestly solicited.

Deposit Account

The Commissioner is hereby authorized to charge any deficiency in payment or credit any overpayment associated with the filing of this correspondence to Deposit Account Number 50-0426.

Respectfully submitted,

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